Device for automatically performing temperature cycles

The invention relates to a device for automatically performing temperature cycles in a plurality of test tubes, each test tube being closed by a closure and containing a predetermined volume of a liquid reaction mixture, which device comprises the following components:

a) a support which has an arrangement of chambers for holding the test tubes, each chamber being suitable for holding the lower portion of a test tube, and the support consisting of a material that has high thermal conductivity, and the support has an upper surface, a lower surface and a cylindrical outer wall, each of the chambers of the support having an opening which is located in the upper surface of the support;

b) a computer-controlled control and regulation device; and

c) means controlled by the control and regulation device for cyclically changing the temperature of the support.

The invention relates especially to a device of that kind which, preferably as an integrated component of an automatic analysis apparatus, is suitable for carrying out the polymerase chain reaction.

A device of the kind mentioned above is described in EP-A-0 236 069 A2. In that known device, the test tubes are in a matrix-like arrangement, which makes it difficult to achieve a uniform temperature for all test tubes. The device constructed in accordance with EP-A-0 236 069 A2 is relatively bulky and its operation requires a relatively large amount of power. It is therefore not suitable for use as an integrated component of a modern automatic analysis apparatus.

Devices of the kind mentioned at the beginning are known as "thermal cyclers". That term is used in the description which follows.

The invention is therefore based on the problem of providing a device of the kind mentioned at the beginning, the dimensions of which are as small as possible and the operation of which requires as little power as possible.

According to the invention, that problem is solved by a device of the kind mentioned at the beginning that is characterised in that the chambers are in a ring-shaped arrangement in the support, and the closure of each test tube can be pierced with a pipetting needle.

The substantial advantages of the device according to the invention are that it has relatively small dimensions and its operation requires a relatively small amount of power, so that it is suitable for use as an integrated component of an automatic analysis apparatus.

<u>Description of an exemplary embodiment:</u>

An exemplary embodiment of the invention will be described below with reference to the accompanying drawings:

Fig. 1	shows, removed from an analysis apparatus, a thermal cycler component 2 which comprises thermal cyclers 18 and 19 according to the invention, the Figure showing the thermal cycler 18 open and a test tube ring 23 removed therefrom;
Fig. 2	is a section through the line II – II in Fig. 1, the thermal cycler 18 being closed;
Fig. 3	is a perspective view of the thermal cycler 18 according to Fig. 1 which has been supplemented by a lifting device 53;
Fig. 4	shows, on an enlarged scale relative to Fig. 2, a section through the thermal cycler in the closed state;
Fig. 5	shows a thermal cycler according to Fig. 4 in the open state;
Fig. 6	is a diagrammatic view of a "master-slave" control means for regulating and monitoring the operating parameters of a thermal cycler according to the invention;
Fig. 7	is a temperature-time graph of a temperature sequence stored in the master processor and the temperatures of the thermal block and of the sample resulting therefrom;

Fig. 8 is a perspective exploded view of the individual parts 92 and 95 of a test tube arrangement 23 which is suitable for use in a thermal cycler according to the invention;

Fig. 9 shows the individual parts 92 and 95 in accordance with Fig. 8 assembled and with the test tubes of the test tube arrangement 23 so formed in the open state;

Fig. 10 shows the individual parts 92 and 95 in accordance with Fig. 8 assembled and with the test tubes of the test tube arrangement 23 so formed in the closed state;

Fig. 11 is a section through a test tube 21 in Fig. 9 with its lid 87 open;

Fig. 12 is a section through a test tube 21 in Fig. 9 with its lid 87 closed;

Fig. 13 is a perspective overall view of an analysis apparatus which comprises, as a component, a thermal cycler component 2 according to the invention.

Thermal cycler

In the description which follows, the term "thermal cycler" is used to denote a device which serves for automatically performing temperature cycles in at least one test tube 21 closed by a closure, which test tube contains a predetermined volume of a liquid reaction mixture.

A thermal cycler according to the invention is described below which is especially suitable as a component of an automatic analysis apparatus for carrying out the polymerase chain reaction. The analysis apparatus is configured, for example, for carrying out immunoassays.

Fig. 1 shows a thermal cycler component 2 removed from an analysis apparatus 1 according to Fig. 13. The thermal cycler component 2 comprises, for example, two identical thermal cyclers 18, 19 and a stand-by position 22. The following description of the thermal cycler 18 applies also to the thermal cycler 19.

The thermal cycler 18 comprises the following components:

a) a thermal block 33, which serves as a support for the test tubes, and which has a ring-shaped arrangement of recesses 27, each recess serving as a chamber for holding the lower portion of one of the test tubes 21;

b) a computer-controlled control and regulation device shown in Fig. 6; and

c) heating and cooling elements, which are controlled by that control and regulation device, as means for cyclically changing the temperature of the thermal block 33.

The thermal block (33) consists of a material having high thermal conductivity. The thermal block 33 is preferably a body made of aluminium or silver. The thermal block (33) has an upper surface, a lower surface and a cylindrical outer wall, each of the recesses (27) of the thermal block (33) having an opening which is located in the upper surface of the support.

As shown in Figures 1 and 3, twelve test tubes 21, for example, are combined to form a test tube ring 23. The test tubes 21 are conical in their lower region and cylindrical in their upper region and are tightly closed by a lid 87. As can readily be seen in Fig. 1 and 3, such a test tube arrangement 23 can be inserted into corresponding recesses 27 in the thermal block 33 of the thermal cycler 18.

<u>Access to the contents of a test tube</u>

The thermal cycler 18 has a hinged lid 28, which has for each recess 27 of the thermal block 33 an opening 29 which allows the closure 87 of the test tube 21 inserted in the recess to be pierced by a pipetting needle. As can be seen from Fig. 2, when the hinged lid 28 is in the closed position each of the openings 29 is aligned with the longitudinal axis 31 of the corresponding test tube 21.

The openings 29 in the hinged lid 28 allow access to the contents of each test tube when the hinged lid 28 is closed. For that purpose, the pipetting needle 32 of a pipetting device (not shown in Figure 3) is introduced through one of the

openings 29, the lid 87 of the test tube 21 is pierced with the pipetting needle 32 and then a certain volume of the liquid contained in the test tube is extracted.

Heat transmission between thermal block and test tube

It can be seen from Fig. 2 that the recesses 27 in the thermal block 33 are matched to the conical region of the test tubes 21, so that the peripheral wall of the test tube 21 can reliably make contact with the inner wall of the recess 27 for the purpose of the best possible heat transmission. In order to increase the thermal reaction rate, precision and homogeneity, the thermal block 33 is mounted, as far as possible thermally insulated, in a housing 34 and has low mass combined with good thermal conductivity.

Heating element in the hinged lid of the thermal cycler

The lid 28 preferably comprises a heating element, for example an electrical resistance heater 52, which serves for heating the closed test tubes arranged in the thermal block 33.

In a first embodiment of the thermal cycler, the electrical resistance heater 52 is used in combination with a Peltier element 36 described below in order to achieve a desired temperature profile (temperature sequence over a certain time period) in the thermal block 33. In such an embodiment, the Peltier element is used as a cooling element or as a heating element depending upon the temperature to be achieved within a temperature profile.

The interaction of the electrical resistance heater 52 with the Peltier element 36 makes it possible to achieve the necessary rapidity of the temperature changes of the thermal block 33 as well as the necessary precision and homogeneity of the temperature distribution. In addition, the action of the resistance heater 52 prevents the formation of any condensation in the lid region of the test tube 21.

Closing and pressing device of the hinged lid of the thermal cycler

The hinged lid 28 preferably comprises a closing and pressing device for securing the closed test tubes 21 arranged in the thermal block 33. For that purpose, the hinged lid 28 has a resiliently mounted presser plate 46 which presses each test tube 21 with a defined force into the recesses 27 of the thermal block 33. Recesses 47 for receiving the cap-shaped lids 87 of the test tubes 21 as well as penetration openings 48 for the pipetting needles 32 are provided in the presser plate 46 coaxially with respect to the test tubes 21. As resilient element, a corrugated washer 49 can be provided. By means of a securing ring 51, the presser plate 46 is secured against falling out when the hinged lid 28 is open.

The above-mentioned resistance heater 52 is preferably contained in the resilient presser plate 46.

Peltier element as cooling or heating element

As shown in Fig. 2, a thermal cycler 18 according to the invention preferably comprises at least one Peltier element 36 as part of the means provided in the thermal cycler 18 for cyclically changing the temperature of the thermal block 33. One heat transfer surface 37 of the Peltier element 36 is in thermal contact over a large surface area with the lower surface of the thermal block 33 and its other heat transfer surface 38 is in thermal contact over a large surface area with a cooling body 39 for dissipating heat. The cooling body 39 is preferably made of aluminium or copper. A switchable fan 45 is provided for heat dissipation.

The Peltier element 36 shown diagrammatically in Fig. 2 is especially an arrangement of such elements.

In the above-mentioned first embodiment of the thermal cycler, the Peltier element 36 is used as a cooling element or as a heating element. That mode of operation of the Peltier element 36 and its interaction with the electrical resistance heater 52

make it possible to achieve the required temperature of the thermal block within a temperature profile.

To prolong the service life of the Peltier element 36, the Peltier element 36 is protected against thermodynamically caused mechanical stress peaks, preferably by its being held pressed against the thermal block 33 by means of a central, spring-biased fixing means. For that purpose, the Peltier element is clamped resiliently between the heat transmission surfaces of the thermal block 33 and of the cooling body 39. To that end, the contact surface of the cooling body 39 is pressed against the Peltier element 36, for example by means of a compression spring 41. The spring bias can be adjusted by means of an adjusting screw 42, spring collar 43 and a ball joint 44 which further increases the degrees of freedom of the cooling body 39.

Peltier element exclusively as a cooling element

In a variant of the exemplary embodiment described herein, the Peltier element 36 is used exclusively as a cold-generating element, that is to say only as a cooling element. This prolongs the service life of the Peltier element.

Additional heating element around the thermal block

In a second embodiment of the thermal cycler, the thermal cycler preferably additionally comprises an electrical resistance heater 35, which is arranged around the thermal block 33 and along the periphery of its cylindrical, outer wall. When such an additional heating element is used in the thermal cycler, the Peltier element 36 is used only for cooling. This brings the advantage that the Peltier element is relieved of thermally induced mechanical stress and also contributes to prolonging the service life of the Peltier element in the thermal cycler.

Means for identifying a marker on the test tube ring

The thermal cycler 18 also preferably comprises means for identifying a marker on the test tube arrangement 23, for example a marker in the form of a vertically oriented flag 25. The flag 25 cooperates with a detection device 26 within the thermal cycler 18 in order that the presence of the test tube ring 23 in the thermal cycler 18 can be detected. The detection device 26 is, for example, a light barrier. The flag 25 also allows the test tube arrangement 23 to be placed in the thermal block 33 in only one position. That one position, combined with numbering of the closures of the test tubes, also facilitates unambiguous sample/patient allocation.

The test tube arrangement 23 also has a tab 24 which serves, for example, as a support surface for data relating to the sample contents of the arrangement 23, which data are present, for example, in the form of a bar code.

Lifting device

The changes in temperature and the action of the spring 49 cause the conical regions of the test tubes 21 to adhere to the walls of the recesses 27 of the thermal block 33. The non-positive connection thereby created makes it difficult to remove the test tubes 21 from the thermal cycler 2. For that reason, in the embodiment according to Fig. 3 to 5 there is proposed a lifting device 53 which makes it considerably easier to remove the test tube ring 23 from the thermal block 33.

As can be seen from Figures 3-5, the lifting device 53 comprises a rocker 55 which acts as an ejector lever. The rocker 55 is connected at one end to a hinge of the hinged lid 28. The rocker 55 is free at the other end. The lifting device 53 also comprises an ejector disc 58 which is concentric with the axis of rotational symmetry of the thermal block 33 on which the rocker 55 is arranged. The ejector disc 58 has an arrangement of recesses 61 around its periphery which serve for removal of the test tube ring 23 from the recesses 27 of the thermal block 33.

As can be seen from Fig. 3, the rocker 55 is guided on the pivot axis 54 of the hinged lid 28. At its pivot axis end, the rocker 55 has two tongues 56 having openings 57 in which the pivot axis 54 engages. The ejector disc is screwed onto the rocker 55. The ejector disc 58 has semi-circular recesses 61 around its peripheral edge 59 which align accurately with the recesses 27 in the thermal block 33 and the cylindrical regions of the test tubes 21 which are inserted into the recesses 27 (Fig. 5). The peripheral edge 59 of the ejector disc 58 accordingly engages under the inner flange-like region 62 of the test tube ring 23 or the flanges of the test tubes 21. The shape and function of the opening 57 in the tongues 56 of the rocker 55 in conjunction with the pivot axis 54 of the hinged lid 28 as well as of a control pin 63 mounted on the hinged lid 28 at distance e, which pin 63 likewise engages in the opening 57, can be seen in Figures 4 and 5. When the hinged lid 28 is closed, the lifting device 53 as yet has no function. When the hinged lid 28 is opened, once a certain opening angle has been reached the pin 63 makes contact with a control surface 64 of the opening 57 and effects pivoting of the rocker 55 about point P, which results in the sample containers 21 being lifted out. The tilting movement of the rocker 55 about the point P and the increasingly sloped position of the ejector disc 58 has the effect that the breakaway torque forces associated with the individual test tubes 21 come into effect staggered in time, so that the test tubes 21 are gradually released from their recesses 27. The force applied and the stress exerted on the material is thus kept at a low level and increases the ease of operation.

Control and regulation of the thermal cycler

A control and regulation device of a thermal cycler 18 according to the invention by means of master-slave processors 72, 73 is shown diagrammatically in Fig. 6.

The temperature of the presser plate 46 of the hinged lid 28, of the thermal block 33 and the surrounding area is detected by means of temperature sensors 65, 66, 67 and supplied to the slave processor 73 by way of a temperature interface 68. In the master processor 72 (interface to the user) there are input *inter alia* the

desired values for temperature, the desired values for time, the number of temperature cycles and the rate of the heating and cooling processes.

It is possible to select and start from predetermined, stored temperature/time profiles. The input is effected *via* a keyboard 16 or some other interface. Those data are supplied to the slave processor 73 which controls, by means of regulators 69, a power controller 71 which in turn regulates the energy supply to the heating elements 35, 52 and to the Peltier element 36. The feedback messages (actual values) are supplied *via* the slave processor 73 to the master processor 72 where they are processed or displayed to the user. In that way, the user is informed as to the instantaneous sample temperature, the temperatures already reached with time data and the temperatures still to be reached with time data.

The operating state of the system is constantly monitored and logged. Errors that cannot be eliminated by the system itself result in automatic switching-off or an error message.

The temperature of the sample is calculated from the temperature of the thermal block 33. For that purpose, the transfer function from the sample chamber to the sample in the test tube 21 is determined. That function is basically a low pass (filter?) with dead time.*

On the basis of suitable regulation algorithms (scanned systems), there is calculated in each case the control value that is necessary to bring the temperature of the sample into line with the preset desired value temperature. Those calculations are carried out using a signal processor. The calculated control value is supplied to the power controller 71 in the form of a pulse width. The power controller 71 is, for example, a power FET with a suitable protection and interference suppression circuit.

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* I'm really not sure what the last two sentences mean – please get back to me if this aspect is important!

The above-described control and regulation allows the use of the thermal cycler in order to heat and to cool samples in a test tube ring inserted in the thermal cycler in accordance with specific temperature profiles. The temperature profiles are defined by plateau temperatures of defined duration, and the gradient which defines the time at which a plateau temperature has to be reached. It is a condition that all samples in the thermal cycler have the same temperatures at the same time.

Fig. 8 shows, for example, temperature sequences from a cycle process. Curve A shows the temperature sequence at the thermal block 33, curve B shows the temperature sequence of the liquid in the reaction vessel 21. Using the thermal cycler it is possible to set temperatures between 40 and 98°C. Typically the lower temperatures lie between 50 and 60°C and the upper temperatures between 90 and 96°C. When the middle temperature is used, it is around 72°C. The heating/cooling rate achieved using the thermal cycler is 1°C per second. A typical cycle has a duration of 120 seconds. When the temperatures in question have to be maintained for longer than 10 seconds, the cycle duration is lengthened accordingly.

Test tubes

As can be seen especially from Figures 8 to 12, the test tubes 21 have a conical lower region 82 and a cylindrical upper region 81. The conical, lower region 82 of the test tube 21, in which the sample to be subjected to thermal treatment is located, is provided with a thinner wall thickness than the upper, cylindrical region 81 on account of the better heat transmission. As can be seen from Fig. 3, the lower, conical region 82 of the test tube 21 can be fitted exactly into a correspondingly shaped recess 27 in the thermal block 33 of the thermal cycler 18, so that the conical inner wall of the recess 27 in the thermal block 33 makes full contact with the conical outer wall 85 of the lower region 82 of the test tube 21 and accordingly ensures the best possible transfer of heat.

The opening 86 of the test tube 21 is tightly closable by means of a lid 87. The lid 87 can be pierced with a pipetting needle 32 for the purpose of removing sample material.

In order to increase efficiency and to facilitate the handling of the test tubes 21, a plurality of, for example twelve, test tubes are combined to form a unit, preferably in a circular configuration to form a test tube ring, and the lids 87 are mounted captively by means of a film hinge 91.

In an especially advantageous manner, the test tube arrangement 23 is of two-part construction. One part 92 consists of test tubes 21 arranged at equal angular distances from one another, the test tubes being joined at their flange-like, opening-side portions of increased diameter 93 by means of thin webs 94 to form a circle. In order to achieve radial flexibility of the test tube ring 92, which is advantageous for assembly with the other part 95, the webs 94 are V-shaped. The part 92 is preferably produced from polypropylene (PP).

The other part 95 of the test tube arrangement 23 has circular rings 97 arranged in a circle and joined to one another by webs 96, the internal diameter of which rings 97 is identical to the external diameters of the cylindrical regions 81 of the test tubes 21 and the centre points of which are in alignment with the longitudinal axes 98 of the test tubes 21. The webs 96 are V-shaped to provide radial elasticity. Integrally formed with the circular rings 97 are radially outwardly pointing film hinges 91 which each carry a closure lid 87 at the end. The part 95 is preferably likewise produced from polypropylene (PP).

Two extension pieces 99 and 101 are formed integrally with the other part 95, which extension pieces are displaced by half the angular pitch of the circular rings 97, project radially outwards and are arranged diametrically opposite one another. One extension piece 99 has a horizontal surface 102 on which, for example, the data relating to the samples in the test tubes 21 are applied by means of a bar code. The other extension piece 101, in the form of a vertically oriented flag, cooperates with a detector 26, for example a light barrier, in the thermal cycler 18

(see Fig. 1). By means of such a device, the test tube arrangement 23 is forced to be inserted into the thermal cycler 2 in a defined way.

To render the sample number better visible to the operator, the sample number can be applied to the lid tabs of the test tube.

When the two parts 92, 95 of the test tube arrangement 23 (Fig. 9) are assembled, the flanges 93 of the test tubes 21 of the one part 92 make contact with the upper side 104 of the circular rings 97 of the other part 95. Because of the close fit between the cylindrical region 81 and the circular ring 97, the test tube arrangement 23 is relatively securely premounted and can be filled with the samples in question. The lids 87 are then folded over and the cylindrical extension piece thereof 105 is held sealingly in the opening 86 of the test tubes 21. (Fig. 10).

The ribs 94, 96 that are provided in the above-described construction of the test tube arrangement 23 render the test tube arrangement flexible so that the test tubes 21 can very easily be introduced into the recesses 27 in the thermal block 33, which, when a test tube arrangement 23 is of rigid construction, can be difficult even if the dimensions of the thermal block and the test tube arrangement differ only very slightly.

By virtue of the two-part construction of the test tube arrangement 23, extremely economical use is made of materials and when, advantageously, materials (plastics) having different properties are also used, operation is purpose-optimised, which is important in the case of disposable articles (the test tube arrangement is discarded after use).

<u>Analysis apparatus with thermal cycler</u>

Fig. 13 shows an analysis apparatus 1 which is configured, for example, for carrying out immunoassays.

In order to increase the volume of the substances to be analysed contained in the samples to above the detection limit for the subsequent analysis processes, there is provided as an integrated component in the analysis apparatus a thermal cycler component 2 which comprises thermal cyclers 18 and 19 according to the invention described above, by means of which a DNA amplification process can be carried out by the use of the polymerase chain reaction.

In order to increase the efficiency of the analysis apparatus, that is to say to process the greatest possible number of samples per unit time, the prepared number of samples should be matched to the subsequent process times in such a manner that no dead times arise. For example, this can be brought about by the two independently operating thermal cyclers 18, 19, each of which can accommodate twelve test tubes 21, as well as by two "stand-by" stations 22, each of which can likewise accommodate twelve test tubes 21, which are removed from one of the thermal cyclers 18, 19 at the end of the process carried out therein.

Furthermore, the analysis apparatus 1 comprises all further devices for carrying out the above-mentioned immunoassays, for example two racks 3, 4 with reagents on a shaking platform 5, a rack 6 with further reagents, three racks 7 with disposable reaction vessels 8, a heat-controllable incubator 9 in which the reaction vessels 8 are inserted, a washing device 11 and a photometer device 12 for ascertaining the test result.

<u>Transfer head of the analysis apparatus</u>

The transfer of samples and reagents as well as the transfer of the reaction vessels is facilitated by a transfer head 13 movable in the x-y coordinate system, which transfer head 13 has a pipetting device 14 and a reaction vessel gripper 15, both movable in the z-direction.

After the DNA amplification has been carried out in the test tubes 21 contained in the thermal cyclers 18, 19, the pipetting device 14 is used to remove sample volumes from the test tubes 21 and deposit them in reaction vessels 8 which are

arranged in the racks 7. The sample volumes deposited in the reaction vessels 8 are investigated in the immunoassays carried out with the analysis apparatus.

Control unit of the analysis apparatus

All operations to be carried out are controlled and coordinated by a central control unit (not shown) of the analysis apparatus. A user interface 16 or keyboard for inputting process parameters, as well as a display for displaying process states is shown diagrammatically. The sample data, which are applied to the test tubes, for example, by means of a bar code, can be read into a memory by means of a manually guided stylus or scanner 17. Interfaces for a printer *etc.* (not shown) are provided.

Patent claims

1. A device for automatically performing temperature cycles in a plurality of test tubes, each test tube being closed by a closure and containing a predetermined volume of a liquid reaction mixture, which device comprises the following components:
   a) a support which has an arrangement of chambers for holding the test tubes, each chamber being suitable for holding the lower portion of a test tube, and the support (33) consisting of a material that has high thermal conductivity, and the support has an upper surface, a lower surface and a cylindrical outer wall, each of the chambers (27) of the support (33) having an opening which is located in the upper surface of the support;
   b) a computer-controlled control and regulation device; and
   c) means controlled by the control and regulation device for cyclically changing the temperature of the support,

   which device is characterised in that the chambers (27) are in a ring-shaped arrangement in the support (33), and the closure (87) of each test tube (21) can be pierced with a pipetting needle.

2. Device according to claim 1, characterised in that the means for cyclically changing the temperature of the support contain as cold- and heat-generating element at least one Peltier element (36) which is thermally connected to the lower surface of the support.

3. Device according to claim 1, characterised in that the means for cyclically changing the temperature of the support contain at least one Peltier element (36) which is used exclusively as cold-generating element and which is thermally connected to the lower surface of the support.

4. Device according to claims 2 or 3, characterised in that the at least one Peltier element (36) is pressed against the support (33) by means of a central spring-biased fixing means (41, 42, 43, 44), which fixing means

contains a spring (41) compressed by screw (42), the tension of which spring can be adjusted with the screw.

5. Device according to claim 1, characterised in that it also comprises a hinged lid (28) which contains a heating element (52) which serves for heating the closed test tubes (21) arranged in the support (33) and which has for each chamber (27) an opening (29) which allows the closure (87) of the test tube (21) contained in the chamber (27) to be pierced with a pipetting needle.

6. Device according to claim 5, characterised in that the hinged lid (28) comprises a closing and pressing device for securing the closed test tubes (21) arranged in the support (33).

7. Device according to claim 1, characterised in that it also comprises a heating element (35) which is arranged around the support and along the periphery of its cylindrical outer wall.

8. Device according to claim 1, characterised in that it also comprises means for identifying a marker (25) of an arrangement of test tubes (21).

9. Device according to claim 5, characterised in that it also comprises a lifting device for facilitating removal of the test tubes (21) from the chambers (27) in the support (33), which lifting device comprises an ejector lever (55) which is connected at one end to a hinge of the hinged lid (28) and is free at the other end, and an ejector disc (58) which is concentric with the axis of rotational symmetry of the support (33), is fixed on the ejector lever (55) and has around its periphery an arrangement of recesses (61) which serve for removal of the test tubes (21) from the chambers (27).